

Study of a passive haemagglutination test for gonorrhoea

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In recent years the rising incidence of gonorrhoea in Europe and America has stimulated research in immunological methods for its diagnosis. Most serological techniques have been applied; bacterial agglutination (Wilson, 1954), passive haemolysis (Maeland, 1966), complement-fixation (Magnussen and Kjellander, 1965), passive haemagglutination (Chanarin, 1954; Logan, Cox, and Norins, 1970), latex-agglutination (Watt, Ward, and Glynn, 1971), Bentonite agglutination (Wallace, Diena, Yugi, and Greenberg, 1970), microprecipitation (Reising and Kellogg, 1965; Chacko and Nair, 1969), lecithin-cholesterol particle agglutination (Reising, 1971), and fluorescent antibody staining methods (Ovčinnikov, 1963; Danielsson, 1965a).

Sensitizing antigens used in these tests have been in general unfractionated soluble extracts of gonococci ruptured by various means. Some tests have utilized phenol-extracted antigens. Many workers have reported interference in their tests by cross-reacting antibody to other bacterial species (Reyn, 1943, 1949; Danielsson, 1965b; Logan, Cox, and Norins, 1970). The use of phenol-extracted antigen in some tests has given rise to fewer cross-reactions, although this is accompanied by a loss of sensitivity.

That qualitative differences in antigen specificity exist between *N. gonorrhoeae* strains has been recognized for a long time, and a recent report by Maeland, Kristoffersen, and Hofstad (1971) has revealed that lipopolysaccharide specificity varies from strain to strain. This makes the selection of strains for extraction of diagnostic antigens a difficult task.

In all reports of diagnostic tests for gonorrhoea, single strains were used as the source of antigen. The present report describes the performance of a passive haemagglutination test for gonorrhoea (GCHA), using as sensitizing antigen a pool of phenol-extracted

antigens from ten isolates of *N. gonorrhoeae*. By this means it was hoped to take advantage of the greater specificity of the lipopolysaccharide antigen and to increase the sensitivity by using antigen from a variety of serotypes.

Material and methods

Sera were obtained from men and women attending the Lydia clinic at St. Thomas' Hospital. Blood donor sera were obtained from Dr. Rogers of the South London Blood Transfusion Centre.

Neisseria gonorrhoeae isolates were obtained from men attending the same clinic and were used immediately after isolation. Culture was done by seeding isolates on 2 per cent. lysed horse blood agar plates and incubating in a 10 per cent. CO₂/air atmosphere for 18 hrs. Growth was scraped off, washed twice with 0.9 per cent. saline, and lyophilized. Cultures were checked for purity by Gram-staining and identity confirmed by fermentation tests.

Organisms were suspended in 100 ml. phosphate buffered saline (0.02M, pH 7.0, 0.9 per cent. NaCl) and extracted with 90 per cent. aqueous phenol by the method of Westphal, Lüderitz, and Bister (1952). The aqueous phase was dialysed against phosphate buffered saline (PBS) and then incubated at 37°C. for 8 hrs with 0.5 mg. ribonuclease, 0.5 mg. deoxyribonuclease, and 25 mg. magnesium sulphate. This was followed by incubation at the same temperature for 16 hrs with 1 mg. trypsin. The solution was dialysed against PBS and then precipitated by the addition of four volumes of ethanol. The precipitate was re-suspended in 50 ml. PBS and re-extracted with aqueous phenol as above. The final aqueous solution was exhaustively dialysed against PBS and then lyophilized.

Yields varied from 0.75 to 2.1 per cent. and nitrogen values estimated by the Kjeldahl test, from 2.9 to 6.2 per cent.

The antigens from ten isolates were prepared in this way and were pooled, dissolved in PBS to a concentration of 0.25 mg./ml., and stored frozen in aliquots.

Fresh sheep erythrocytes were washed four times by centrifugation with PBS and re-suspended to a packed cell concentration of 10 per cent. To 5 ml. of suspension, 2 ml. of sensitizing antigen (250 µg./ml.) was added, and this was incubated at 37°C. for 1 hr. The suspension was

then washed four times and re-suspended in PBS at a final concentration of 2.5 per cent. packed cells.

Sera were inactivated at 57°C. for 30 min. and then doubling dilutions using PBS were made in a WHO pattern agglutination tray from 1 in 2 to 1 in 1,024. The volume of each dilution was 0.2 ml. To each dilution 1 drop (0.05 ml.) of sensitized sheep cells was added. After shaking, the plates were incubated at 37°C. for 30 min and then at 4°C. for 20 hrs. Agglutination end points were read by shaking the plates and observing the highest dilution in which significant clumping could be seen. Known positive serum and PBS controls were included on each plate.

Results

Sera from the following were assayed: blood donors, children with various non-sexually transmitted diseases, men and women in whom gonorrhoea had been diagnosed by culture and/or smear, antenatal patients, women attending the special clinic for non-specific genital infections, and men attending the special clinic for non-specific urethritis. The results are given in Table I. Positive titres in men and women with gonorrhoea ranged up to 1:1,024, whereas with blood donor sera the highest titre obtained was 1:256. Mean titres of sera in the various groups tested are included in Table I.

TABLE I *Haemagglutination titres in control and trial groups*

Source	No. examined	1:128 or greater	Mean titre
Children	17	0	1:18
Blood donors Total	119	14.3	1:51
Male	70	21.4	1:62
Female	49	8.1	1:36
Antenatal clinic patients	50	20.0	1:66
Women with N.S.G.I.	100	24.0	1:80
Men with N.S.U. ^b	38	18.1	1:70
Women with gonorrhoea ^a	100	82.0	1:220
Men with gonorrhoea ^a	100	51.0	1:109

^aDiagnosed by culture and/or smear.

^bChlamydia negative.

TABLE II *Haemagglutination titres of female contacts of cases of gonorrhoea*

Serum taken	No. examined	Titre 1 in 128 or greater
On first visit	200	75.0
1 week later	86	70.9
3 months later	17	47.1

Gonorrhoea was diagnosed in 76.5 per cent. by culture and/or smear.

Infected women were tested on their first visit, on their second visit about 1 week later, and on a third visit some 3 months later. A comparison of the titres on these three visits is given in Table II.

When a series of 200 female contacts of men with gonorrhoea diagnosed at this hospital was investigated 58.5 per cent. were positive both by culture and GCHA, 7 per cent. were negative by both procedures. 18 per cent. were positive by culture but not by GCHA, and 16.5 per cent. were negative by culture but positive by GCHA.

For comparison, sera from control and trial groups were tested under the same conditions but using sheep cells sensitized with antigen derived from a single strain of *N. gonorrhoeae*. The results are given in Table III. The difference in sensitivity between single and multiple strain derived sensitizing antigen is quite marked, being statistically significant at the $P = 0.005$ level.

TABLE III *Haemagglutination titres in control and trial groups using single strain sensitizing antigen*

Source	No. examined	Titre 1 in 128 or greater
Blood donors:		
Male	50	18.0
Female	36	16.7
Men with gonorrhoea	50	38.0
Women with gonorrhoea	100	64.0

Several sera were stored at -10°C. for 6 months and re-tested; in every case the titre was unchanged. Variations in the methodology of the test were tried and the temperature of incubation was found to be uncritical between 4° and 37°C., provided the period concerned exceeded 15 hrs. Haemolysis occurred in lower serum dilutions if they were not inactivated and this caused difficulty in reading low titre sera. No differences in titre were found between normal and inactivated sera.

Absorption of sera with washed sheep cells gave a reduction in titre of two dilutions, occasionally of one or three. Agglutination of unsensitized sheep cells by fifty sera from women with proven gonorrhoea gave titres from 1 in 2 to 1 in 64, only three sera giving the highest dilution and seven the next highest. No attempt was made to correct for this heterophil reactivity, but as the level was fairly constant only small inaccuracies will be present.

Discussion

The test described was of greater sensitivity than previously reported tests using phenol-extracted antigens. Evidence of a significant difference between

single and multiple strain derived antigen tests suggests that the improvement is due to the use of several strains in preparing antigen for sensitization. The reason for this may well be the existence of different *N. gonorrhoeae* serotypes reported by Maeland and others (1971).

In agreement with many previous reports the percentage of positive titres amongst control sera was quite high and precludes the use of the test for diagnostic screening purposes. Whilst a part of the positives in this group might indicate undetected or treated gonorrhoea, it is unlikely that this could be the case with all such 'false positives'. Cross-reactions are known to occur between *Neisseria* species and past episodes of meningitis, or carriage of *N. catarrhalis* and other *Neisseria* spp. could be responsible in large part for the observed reactions. In addition interference from heterophil antibody is suggested by the results of absorption of sera with sheep cells. Similarities in chemical structure of lipopolysaccharides, heterophil antigens, and blood group mucopolysaccharides are well known and it is probably in this area that the explanation for the cross-reactions which beset gonococcal serology will lie.

It was not possible to obtain a reliable estimate of the post treatment decline in antibody titre, but of the seventeen women who attended 3 months after their first visit eight still had positive titres. None, to their knowledge, had contacted gonorrhoea in the intervening period. Extrapolation of this insufficient data gives a half life for antibody of 4 to 5 months. This means that the test cannot distinguish between active infection and an infection which occurred up to one year or more previously, depending on the amount of antibody produced.

Long persistence of antibody has been found in other diseases, where studies of the classes of antibody molecules present during and after the course of infection have made reliable diagnostic tests possible. Such a study of gonococcal antibody would be of great interest.

Summary

Sera from patients with gonorrhoea and from control groups were investigated by a passive haemagglutination test in which sensitizing antigen was prepared from ten isolates of *N. gonorrhoeae* by the phenol/water method. Positive titres (1 in 128 or greater) were found in 82 per cent. of women and 51 per cent. of men with active gonorrhoea. In adult control groups the frequency of positive titres ranged from 8.1 and 24 per cent. An identical technique

using sensitizing antigen from a single isolate of *N. gonorrhoeae* was considerably less sensitive.

Persistence of high agglutination titres in women after treatment was demonstrated and the inability of the test to distinguish active from past infection emphasized.

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Étude d'un test d'hémagglutination passive dans la gonococcie

SOMMAIRE

Des sérums de malades atteints de gonococcie et de groupes de sujets témoins furent soumis à un test d'hémagglutination passive dans lequel l'antigène sensibilisant avait été préparé à partir de 10 isolements de *N. gonorrhoeae* par la méthode phénol/eau. On trouva des titres positifs (1/128 ou plus) chez 82 pour cent des femmes et chez 51 pour cent des hommes présentant une gonococcie en activité. Dans les groupes d'adultes témoins, la fréquence de titres positifs alla de 8,1 à 24 pour cent. Une technique identique, utilisant un antigène sensibilisant provenant d'un seul isolement de *N. gonorrhoeae*, a été considérablement moins sensible.

On constata la persistance de titres d'agglutination élevés chez les femmes après traitement et l'on souligne que ce test est incapable de faire la distinction entre une affection active et une infection passée.